

SHORT COMMUNICATION

Hermit crabs (*Pagurus bernhardus*) use visual contrast in self-assessment of camouflage

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ABSTRACT

Animals can make use of camouflage to reduce the likelihood of visual detection or recognition and thus improve their chances of survival. Background matching, where body colouration is closely matched to the surrounding substrate, is one form of camouflage. Hermit crabs have the opportunity to choose their camouflage independently of body colouration as they inhabit empty gastropod shells, making them ideal to study their choice of camouflage. We used 3D-printed artificial shells of varying contrasts against a grey substrate to test whether hermit crabs prefer shells that they perceive as less conspicuous. Contrast-minimising shells were chosen for Weber contrasts stronger than -0.5 . However, in looming experiments, animals responded to contrasts as weak as -0.2 , indicating that while they can detect differences between shells and the background, they are only motivated to move into those shells when the alternatives contrast strongly. This suggests a trade-off between camouflage and vulnerability introduced by switching shells.

KEY WORDS: Colouration, Background matching, Crystallisation, Vision

INTRODUCTION

Background-matching camouflage is a common trait amongst animals; this form of cryptic body colouration has the goal of reducing the likelihood of an animal's visual detection by predators. While the majority of species are constrained by their external body patterning, there are many that have evolved the ability to dynamically tune these patterns, such as cephalopods, chameleons and some fish (e.g. Hanlon, 2007; Stuart-Fox et al., 2006; Smithers et al., 2017). However, colouration on its own is insufficient to avoid detection and cryptically coloured animals must also position themselves in the environment that they are best adapted to match (e.g. Kettlewell and Conn, 1977; Gillis, 1982; Tyrie et al., 2014; Marshall et al., 2016).

One group of animals with an unusual opportunity for behavioural crypsis is the hermit crabs, which inhabit empty gastropod shells to protect their soft abdomens and to provide a mobile shelter. As these shells almost completely cover their bodies (apart from the eyes and part of the legs), hermit crabs have the potential to change their colouration via choice of shell over

relatively short periods of time; although, as these crabs are thought to possess only a single spectral class of photoreceptor (Stieve, 1960; Cronin and Forward, 1988), changes in their own colouration perceptible to these species are probably limited to shell shade and patterning. The behavioural aspects of shell choice have been well studied and various shell properties influence the choice of shell, including the weight, density, size, scent and shade (Lancaster, 1988, and references therein). Self-selection of camouflage is typically observed as the choice of appropriate backgrounds by a freely moving animal: behavioural crypsis. This choice is generally presumed to be driven by the animal's intuitions about the match between its own body colouration and the background (e.g. Kettlewell and Conn, 1977; Gillis, 1982; Merilaita and Jormalainen, 1997; Marshall et al., 2016), provided other factors, such as the thermal properties of the background (Ahnesjö and Forsman, 2006), are controlled for. In most cases, direct control of the degree of similarity between an animal and its background is not possible, as natural colouration varies even within phenotypes, and experimental alteration of an animal's colouration does not necessarily alter its instinctive camouflaging behaviour (e.g. Gillis, 1982).

Previous experiments have shown that hermit crabs select shells and backgrounds dependent on their contrast (as subjectively estimated by the experimenters). Reese (1963) allowed *Pagurus samuelis* without shells to approach 'dark' or 'light' shells situated within glass containers so as to isolate them in terms of tactile and olfactory cues, but leaving them visible. In this case, the animals preferred those shells of the opposite shade to the background. Partridge (1980) conducted experiments with *Pagurus hirsutiusculus* occupying shells painted 'black' or 'white', and showed that black shells are preferred (though the author makes no note of the substrate in this case) and that substrates of similar shade to an occupied shell are preferred. Most recently, Briffa et al. (2008) showed that common hermit crabs (*Pagurus bernhardus*) select black shells on a black substrate and yellow shells on a yellow substrate, this behaviour being suppressed in the simulated presence of a predator. Gravel et al. (2004) also demonstrated that long-clawed hermit crabs (*Pagurus longicarpus*) would accept artificial shells produced via stereolithography in the absence of genuine mollusc shells.

Clearly, it is a common strategy among hermit crabs to choose shells that minimise their visibility against the background but there is currently no understanding of how this behaviour is mediated by vision. In this investigation, we sought to develop an understanding of how this camouflaging behaviour is adjusted depending on an animal's perceived contrast. We used models of *P. bernhardus* vision to calculate the perceived contrast of targets used in two types of behavioural experiment. In the first, hermit crabs chose between shells of various contrasts to the background and in the second, looming startle stimuli were presented on a computer monitor to examine hermit crabs' perception of contrast during a different

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visual task. For our behavioural crypsis experiment, we used 3D printed artificial shells, all of which were constructed using the same design and from the same material. In combination with the roughened polythene base that made up the substrate in these experiments, these artificial shells permitted control over the visual similarity between the animal (when inhabiting a shell) and its background, as well as over potentially confounding factors such as shell shape, density and odour (Mesce, 1982).

MATERIALS AND METHODS

Animals

Animals [*Pagurus bernhardus* (Linnaeus 1758)] were collected from rockpools at Hannafore Point, Looe, Cornwall, UK. Individuals were identified and sexed by morphological criteria (Lancaster, 1988). Only males were used in this study as gravid females demonstrate a reluctance to switch shells (Briffa et al., 2008). Animals were housed in artificial seawater (TropicMarin Salt, Wartenberg, Germany) maintained at 18°C and 35–40ppt salinity. Lighting was by broadband white LED (PowerLED, Aldermaston, UK) (Fig. 1D) on a 12 h:12 h light:dark cycle.

3D-printed artificial shells

Artificial shells were designed using parametric equations (Cortie, 1993) in Matlab (MathWorks, Natick, MA, USA) (Fig. 1A). Digital 3D designs were prepared in Blender (Blender Institute, Amsterdam, The Netherlands) and MakerBot Desktop (Stratasys, Eden Prairie, MN, USA). Shell models were printed in white polylactic acid (PLA; RS Components, Corby, UK) using PVA filament (TechnologyOutlet, Leicester, UK) as a support material. The 3D printer was a Flashforge Creator Pro dual-material printer (Flashforge Corp., Zhejiang, China).

Support material was dissolved away in water and shells were spray painted with primer (Fine surface primer, white; Tamiya America Inc., Irvine, CA, USA), two layers of spray paint (Tamiya) and two layers of transparent matt spray varnish (acrylic, anti-shine matt varnish; The Army Painter, Hoerning, Denmark) (Fig. 1B).

Flat tiles of the same material were also painted in the same manner. All painted materials were soaked in salt water for 1 week and rinsed in freshwater. The reflectance spectra of the paints were measured from tiles using a spectrophotometer (QE65000, Ocean Optics Inc., Dunedin, FL, USA), a UV-Vis light source (DH-2000 Ocean Optics) and reflectance fibre probe (Ocean Optics) (Fig. 1E).

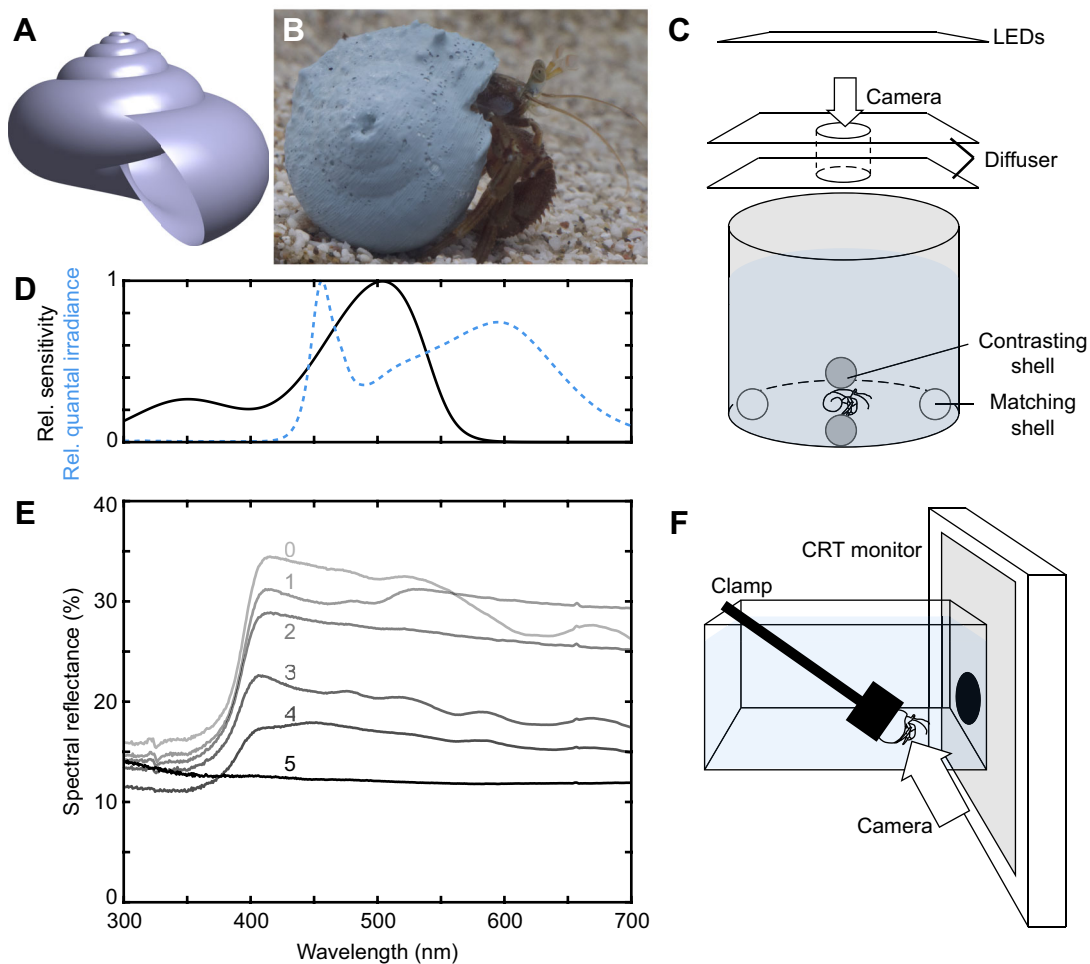


Fig. 1. Methodological aspects. (A) Mathematical model of the shell used to design the 3D printed artificial shells. (B) Hermit crabs were able to inhabit artificial shells – seen here outside the experimental setup in a photographic tank. (C) Setup for the shell choice experiments. (D) Relative sensitivity of the 505 nm peak visual pigment, and relative quantal irradiance of LEDs in the experimental arena. (E) Reflectance spectra of the background-matching (curve 0) and background-contrasting shells (1–5). (F) Setup for the looming experiments. CRT, cathode ray tube.

Shell choice experiments

Shell choice experiments took place inside 12 cm diameter circular polythene containers with roughened bases (Fig. 1C), painted in the same manner as the shells with paint shade number 0 (Fig. 1E) and filled to a depth of 7 cm with seawater.

Animals were removed from their natural shells prior to the experiment using a probe made of fishing line, which was inserted through a hole drilled near the apex of the shell. They were placed individually, without a shell, into the experimental arena inside a vertical opaque cylindrical tube at the centre of the arena to separate them from the shells during a 1 h rest phase before the start of the experiment. Tests were performed six at a time with each crab's container situated within a larger, diffusely illuminated isolated arena. Animals were allowed to choose between four artificial shells: two of the same shade as the background (paint shade 0; Fig. 1E) and two of a darker, contrasting shade (paint shades 1–5; Fig. 1E).

Following removal of the barriers, animals were left undisturbed for 6 h. During this time, the animals' behaviour was monitored via a webcam above each container, programmed to record one image per minute. The first and last choices of shell were recorded from the image sequences.

Six animals were tested on each day and each animal only saw each treatment once. The order of animals and which container position they were tested in was randomised throughout the set of experiments. A total of between 10 and 12 animals were tested in each treatment.

Biases for choices of background-matching over non-background-matching shells were determined via one-tailed binomial tests for a greater than 50% probability of choosing matching shells. Statistical tests were performed in R 3.1.0 (www.R-project.org/) implemented in R Studio 1.0.136 (<http://www.rstudio.com/>).

Looming experiments

To put the shell choice experiments within the wider context of achromatic vision in hermit crabs, a second set of behavioural experiments was performed using 'looming disk' startle stimuli, similar to previous work using this stimulus with marine crustaceans including hermit crabs (e.g. Tomsic, 2016; Shragai et al., 2017). Animals were held using a clamp in a transparent acrylic tank in front of a cathode ray tube (CRT) monitor (S96D, Videoseven, Ingram, Machotron GmbH, Dornach, Germany) (Fig. 1F). The stimulus background was a consistent grey value throughout each session of presentations. Looming stimuli consisted of an expanding dark disc in the centre of the display which subtended a visual angle of 40 deg at their maximum. Preliminary tests showed that startle responses to these stimuli were robust but were prone to habituation following around 10 very high contrast stimuli (Weber contrast approaching -1); therefore, the number of presentations per session was kept below this number. The grey value of the loom was varied randomly between presentations, including a control stimulus of the same value as the background, by definition giving zero contrast. Animals were habituated for 10 min before the start of the presentations and the time between presentations varied randomly between 2 and 3 min. Two different treatments were presented; a brighter and a darker background, with each session consisting of stimuli from only one treatment. A total of 11 animals were used and each animal was tested in two treatments and saw every stimulus. Stimulus contrasts were calculated relative to each background treatment. The experimenter was visually isolated from the experiment by an opaque screen. Animals were observed and recorded using a video camera and monitor external to the experimental setup. Videos were graded as either no response (0) or response (1); responses included:

a stop in normal behaviour or a flick of the antennae, partial retraction into the shell or full retraction.

Results were analysed by a one-tailed binomial test of the proportion of animals that responded against the response proportion at zero contrast. Statistical tests were performed in R 3.1.0 (<http://www.R-project.org/>) implemented in R Studio 1.0.136 (<http://www.rstudio.com/>).

Contrast calculation

Visual contrasts were calculated using relative quantum catch values modelled from measured irradiance and reflection spectra (Fig. 1D, F). In the shell choice experiment, the relative quantum catch of the single spectral class of photoreceptor in the hermit crab (Stieve, 1960; Cronin and Forward, 1988) was calculated for each shade ($i=0-5$) as:

$$Q_i = \int R_i(\lambda)S(\lambda)I(\lambda)d\lambda, \quad (1)$$

where R_i is the reflection spectrum of shade i (Fig. 1E); $S(\lambda)$ is the normalised visual pigment absorbance curve according to an A1 visual pigment template (Fig. 1D) (Govardovskii et al., 2000) peaking at 505 nm (Stieve, 1960); and $I(\lambda)$ is the normalised quantal irradiance spectrum of the illuminating LED light source (Fig. 1D) (Kelber et al., 2003).

Weber contrast, C_i , was calculated as:

$$C_i = \frac{Q_i - Q_0}{Q_0}, \quad (2)$$

where Q_0 is the quantum catch of the background. Contrast was calculated analogously in startle experiments, except with relative quantum catch defined as:

$$Q_i = \int S(\lambda)I_i(\lambda)d\lambda, \quad (3)$$

where $I_i(\lambda)$ is the absolute irradiance spectrum of the monitor at the corresponding greyscale value; Q_0 (in Eqn 2) is the quantum catch of the background and Q_i is the quantum catch of the looming stimulus.

RESULTS AND DISCUSSION

Animals readily occupied the artificial shells and over the course of each experiment moved between the available shells, similar to behaviour observed under natural conditions. For the three lowest contrast levels, the proportions of initial or final choices of shell were not significantly different from the chance level of 0.5 (Fig. 2A). For contrasts stronger than -0.45 , more crabs chose matching than non-matching shells as both their initial and final choices, but only for the final choices was a significantly (Weber contrast= -0.4735 ; binomial test, $P=0.01074$, $n=10$) or marginally significantly (Weber contrast= -0.6285 ; binomial test, $P=0.073$, $n=12$) greater proportion of matching shells chosen (Fig. 2A). No difference was observed in the timing of initial shell choices (median time 278 s; Kruskal–Wallis, $P=0.9565$) or initial choices of matching shells (median time=469 s; Kruskal–Wallis, $P=0.6594$) (see Figs S1–4). In looming experiments, animals responded significantly more often (binomial test, $P<1E-3$, $n=11$) than to the negative control (Weber contrast=0.0) for all stimuli with contrasts stronger than -0.2 , regardless of the background intensity (Fig. 2B). This indicates that although hermit crabs can perceive contrasts weaker than -0.45 , they were not motivated to choose background-

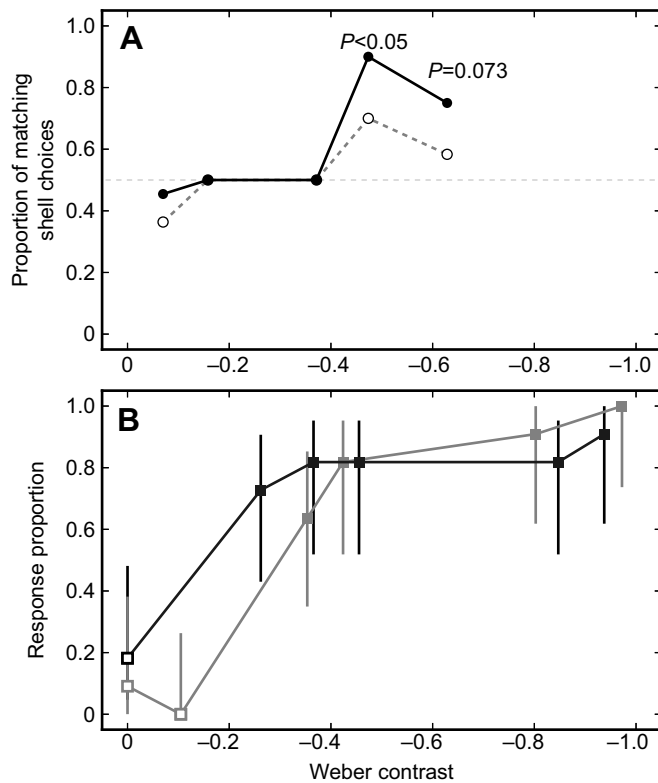


Fig. 2. Behavioural responses. (A) Fraction of choices for shells that matched the background. Open circles: first choices; filled circles: final choices. *P*-values are for a one-tailed binomial test against the chance level (0.5). (B) Proportion of responses in the startle experiments. Black: darker screen; grey: lighter screen. Filled symbols: significantly greater probability of response than with the control (Weber contrast=0) at the 5% level according to a binomial test; open symbols: non-significant. Weber contrast values are negative as all shells and stimuli were darker than the background. Error bars show 95% Wilson score intervals.

matching shells unless the visual contrast of the non-matching alternatives was relatively high.

Previous studies have demonstrated the propensity of hermit crabs to choose shells or substrates so as to reduce their contrast. In the study by Partridge (1980), shells and backgrounds were painted black or white; no measurements of these shades were reported, but it can be safely assumed that these were of relatively high contrast to the hermit crabs' vision. In the work of Briffa et al. (2008), naturally coloured shells and sand substrates were photographed and Michelson contrast was calculated. Weber and Michelson contrasts are not easily compared, and uncalibrated camera images do not give good estimates of perceived contrast from the perspective of non-human visual systems (Stevens et al., 2007). However, the shells in that study were probably also of high contrast.

Here, our results corroborate the finding that given the option between highly contrasting or minimally contrasting shells, hermit crabs select shells that match the background. The results also allow us to quantitatively estimate the extent to which hermit crabs are motivated to perform this behaviour.

There are limitations in making comparisons between shell choice and startle experiments and differences in the contrast sensitivity of behavioural responses may be context dependent. For example, startle responses are driven by motion cues whereas shell choice is a slow response to a static stimulus (Tomsic, 2016).

In conclusion, hermit crabs prefer shells that match the background closely in reflectance, although they are not motivated to switch to a

matching shell below a certain degree of contrast, even though they are seen to be sensitive to lower contrast values. This indicates that they make an assessment of the trade-off between the risks of moving between shells and the benefits of improved camouflage.

Hermit crabs present interesting study species in which to examine the concept of self-selection of camouflage, as they not only choose their substrate but also have both the opportunity and the capacity to maximise their crypsis behaviourally. The synthetic shell paradigm presented here controls for other aspects of shells that can influence choice, such as scent, size and weight, allowing more powerful assessment of the visually guided components of behavioural crypsis, and may be used in the future to test other aspects of self-selected camouflage including chromatic information and patterning.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.W.; Methodology: D.W., I.M.D.; Software: D.W., I.M.D.; Validation: D.W., J.J.F.; Formal analysis: D.W., J.J.F.; Investigation: D.W., S.R., A.B., M.W.; Data curation: D.W.; Writing - original draft: D.W., I.M.D., J.J.F.; Writing - review & editing: D.W., I.M.D., J.J.F.; Visualization: D.W.; Supervision: D.W.; Project administration: D.W.

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Data availability

Data are available from the University of Bristol data repository, data.bris: <https://doi.org/10.5523/bris.3uq1m3hcswj2f2h6yrd94to4p7>.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.173831.supplemental>

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